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(Article begins on next page)

Comparison between principal components and multivariate factor analysis to investigate detailed milk fatty acid profile in sheep.

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ABSTRACT

The fatty acid profile is one of the most important aspects of the nutritional properties of milk. Fatty acid content in milk is affected by several factors as diet, physiology, environment, and genetics. Recently, Principal Component Analysis (PCA) and Multivariate Factor Analysis (MFA) have been used to summarize the complex correlation pattern of the milk fatty acid profile by extracting a reduced number of new variables. In this work, the milk fatty acid profile of a sample of 993 Sarda breed ewes was analysed with PCA and MFA in order to compare the ability of these two multivariate statistical techniques in investigating the possible existence of latent substructures, and in studying the influence of physiological and environmental effects on the new extracted variables. Individual scores of PCA and MFA were analyzed with a mixed model that included the fixed effects of parity, days in milking, lambing month, type of lambing, altitude of flock location, and the random effect of flock nested within altitude. Both techniques extracted the same number of new variables (9) explaining 80% of the total variance. In general, PCA structures were difficult to interpret, with only four PC being associated to a clear meaning. PC1 in particular was the easier to interpret and agreed with the interpretation of the first factor, being both associated to the FA of mammary origin. On the other hand, MFA was able to identify a clear structure of all the extracted latent variables, confirming the ability of this technique, to group FA according to their function or metabolic origin. Key

27 pathways of the milk FA metabolism were identified, as mammary gland *de novo* synthesis, ruminal
28 biohydrogenation, desaturation performed by SCD enzyme, and rumen microbial activity, confirming
29 previous findings in sheep and in other species. Generally, the new extracted variables were mainly
30 affected by physiological factors as DIM, parity and lambing-month; the type of lambing had no
31 effect on the new variables, altitude influenced only one PC and factor. Both techniques were able to
32 summarize a larger amount of the original variance into a reduced number of variables. Moreover,
33 factor analysis conformed its ability in identifying latent common factors clearly related to fatty acid
34 metabolic pathways.

35

36 **Keyword:** fatty acids, principal components, factor analysis,

37

38

INTRODUCTION

39 The interest of the scientific community and of the consumers in the nutritional and health-
40 related properties of milk and dairy products has increased over the last decades. Strategies for
41 improving the milk content of some categories of fatty acids (**FA**) considered beneficial for human
42 health, as PUFA and CLA, have been developed. Most of them rely on feeding management,
43 (Dewhurst et al., 2006; Toral et al., 2010; Nudda et al., 2014) being the diet one of the most important
44 factors affecting milk FA profile (Nudda et al., 2014). However, other factors such as genetics (Carta
45 et al., 2008; Correddu et al., 2019), physiology (De La Fuente et al., 2009), and environment (Sevi et
46 al., 2002) can affect milk FA composition.

47 The elucidation of FA metabolic pathways and the knowledge of factors affecting their
48 regulation are of great interest for improving milk nutritional properties. In particular, the complex
49 phenotypic and genetic correlation pattern existing among individual milk FA hampers the
50 modification of FA profile via feeding and genetic strategies (Cecchinato et al., 2019). Dimension-
51 reduction multivariate statistical methods have been suggested for investigating such a complex
52 correlation network. In particular, principal components analysis (**PCA**) (Fievez et al., 2003;

53 Kadegowda et al., 2008) and Multivariate Factor Analysis (**MFA**) (Conte et al., 2016; Mele et al.,
54 2016; Correddu et al., 2017; Palombo et al., 2020) have been used to highlight common metabolic
55 pathways of FA in ruminant species.

56 Being both based on the factorization of the covariance or correlation matrix, and on the
57 representation of the multivariate system with a lower number of new variables, PCA and MFA
58 appear somewhat similar. However, the way the factorization is carried out differs between the two
59 techniques. PCA is a model-free approach and it is mostly aimed at compressing the variance of the
60 system. PCA is particularly useful when few PC can explain large portion of the variance. On the
61 other hand, MFA starts from a model of the covariance structure of the multivariate system. In
62 particular, the factor model assumes that the covariance of a system could be partitioned in a
63 component shared by all the variables (communality) plus a component specific of each variable
64 (uniqueness). MFA aims at investigating the covariance structure and, in particular, at identifying
65 common latent variables (factors) that generate the quota of shared covariance among the original
66 variables (Krzanowski, 2000; Morrison, 1976). In other words, PCA is more focused on the
67 observations whereas MFA is on the variables, respectively.

68 PCA of cattle milk FA composition was able to assess the relationship between individual
69 milk FA and diet-induced milk fat depression (Kadegowda et al., 2008), and to investigate metabolic
70 relationships among milk FA and to describe their origin (Fievez et al., 2003). PCA has been also
71 used to analyze meat FA profile to differentiate lamb meat according to their origin (Díaz et al., 2005),
72 and to study the relationship between quality traits of carcass and meat of light lamb (Caneque et al.,
73 2014). MFA was successfully used to elucidate relationship between milk FA in dairy cows (Mele et
74 al., 2016; Conte et al., 2016), Sheep (Palombo et al., 2020), and buffaloes (Correddu et al., 2017).

75 The use of the two methods on the same data may provide different and complementary
76 results. In a study of cattle lactation curve traits, for example, PCA was able to extract from the
77 correlation matrix of test day records two new variables related to the whole lactation and to the shape

78 of lactation curve, respectively. On the same data, MFA generates two latent factors related to the
79 first and the second part of lactation, respectively (Macciotta et al., 2006).

80 The aim of this work was to compare results of the use of MFA and PCA in the analysis of
81 milk FA profile in sheep, in order to assess their ability to investigate the complex correlation pattern
82 that exists among these variables.

83

84 MATERIALS AND METHODS

85 *Animals and milk samples*

86 The study was carried out on individual milk samples of 993 Sarda dairy ewes farmed in 48
87 flocks located in the island of Sardinia (Italy). Individual milk samples (one per sheep) were collected
88 from April to July 2014, during the morning milking, by the Provincial Association of Animal
89 Breeders (APA). FA profile of the milk samples was measured using gas chromatography (GC) as
90 previously described (Correddu et al., 2017).

91 *Statistical analysis*

92 Data for a total of 49 individual FA were analyzed with PCA and MFA using SAS
93 PRINCOMP and FACTOR procedures, respectively (SAS Inst. Inc., Cary, NC). The number of
94 principal components (PC) to retain was defined according to the amount of explained variance (\geq
95 80%). In MFA, the number of factors to be extracted was based on their eigenvalue (>1), on their
96 readability in terms of relationships with the original variables and biological meaning, and on the
97 amount of explained variance. Factor interpretation was improved through a VARIMAX rotation.

98 Individual principal component and factor scores for each ewe were calculated and then
99 analyzed with the following mixed linear model:

100
$$y_{ijklmno} = \mu + PAR_j + DIM_k + LM_l + LT_m + ALT_n + F(ALT)_o + e_{ijklmno}$$

101 where $y_{ijklmnop}$ was the principal component or factor score; PAR is the fixed effect of the j -th parity
102 class (eight classes from 1 to >7); DIM is the fixed effect the k -th days in milking interval (five
103 intervals: < 110, 110 to 140, 141 to 170, 171 to 200, >200); LM is the fixed effect of the l -th class of
104 lambing month (1: January; 2: February and March; 3: October and November; 4: December); LT , is
105 the fixed effect of the the m -th type of lambing (two classes: single and multiple birth); ALT , is the
106 fixed effect of the n -th altitude of location of flocks (mountain > 500 mt above the sea level; hill \leq
107 500 and ≥ 200 m a.s.l.; plain < 200 m a.s.l.). Finally, $F(ALT)$ is the random effect of the o -th flock
108 nested within altitude of location; and $e_{ijklmno}$ is the residual term. No effect of the date of the test was
109 included in the model because in most of flocks all samples were collected in the same day.

110

111 **RESULTS AND DISCUSSION**

112 Descriptive statistics of detailed milk FA composition of the 993 samples of sheep milk are
113 reported in Supplementary Table 1.

114

115 ***Principal component analysis***

116 Nine principal components (PC) were able to explain about 80% of the total variance of the
117 system. (Table 1). The variance explained ranged from about 25% for PC1 to about 3% for PC9,
118 respectively. PC scores are often used in dispersion plots to highlight possible clustering or trends in
119 the observations. In the present work, no clear clustering of observations has been detected in the
120 space of the first two PC, even though an overlapped stratification according to parity (Figure 1a) or
121 DIM class (Figure 1b) could be appreciated. In previous studies on milk FA, PCA was effective in
122 grouping animals according to diet they were fed (Correddu et al., 2016; Bernard et al., 2009). PCA
123 was also applied on lamb meat FA to differentiate animals according to their geographical origin
124 (Díaz et al., 2005), or to study the relationship between quality traits of carcass and meat of light
125 lambs (Caneque et al., 2004). Such a different discriminating power among studies could be ascribed
126 to the amount of variance accounted for by the first two PC: 40% in the present study, and 90% in

127 the paper of Correddu et al. (2016), respectively. This is a consequence of the different number of
128 original variables considered (49 and 21, respectively). The larger number of original variables, and
129 therefore of extracted PC (equal to the number of original variables), resulted in the partition of the
130 total variance on a larger number of eigenvalues.

131 The analysis of eigenvector structure is a way for assigning a meaning to the extracted PC in
132 terms of relationship with the original variables. In the present study, the interpretation of the
133 extracted PC on the basis of their eigenvectors (Table 1) was rather difficult. Considering a threshold
134 of ≥ 0.20 (absolute value), half of the FA exhibited coefficients exceeding this value in at least
135 two/three different PC, whereas four FA showed no loading > 0.20 for any extracted PCA (Table 1).
136 This was particularly true for PC4, PC5, PC7, and PC9. An interpretation was attempted for the other
137 PCs.

138 The first PC (PC1) presented highest loadings for most of the short and medium chain FA
139 (negatives), on some iso FA, C18:1*cis*-9 and long chain saturated FA (positives). Most of these FA
140 are totally or partially synthesized in the mammary gland (Chilliard et al., 2000). Therefore, PC1
141 could be considered an index of the activity of this organ. The PC2 had high negative loadings on
142 anteisoC13, C14:0, C16:0, C14:1*cis*-9, C16:1*cis*-9, C18:3*n*-6 and positives on some
143 biohydrogenation products and C18:3*n*-3. The association with FA of different origin and metabolic
144 pathways does not allow to assign a clear meaning to this PC. The only feature shared by FA
145 associated to this PC is their relationship with diet quality, especially with the use of grazing. In dairy
146 cattle (Fievez et al., 2003) the two first PC were mostly associated to FA belonging to four groups.
147 Two included FA that originate in the mammary gland from *de novo* synthesis or desaturase activity;
148 the other two consists of FA produced in the rumen from the biohydrogenation activity or from
149 microbial synthesis.

150 The PC3 presented high positive loadings for C15:0 and C17:0, and negative for several
151 positional isomers of trans C18:1 and on C18:1*cis*-12, respectively. This PC could be related to the
152 FA biohydrogenation processes occurring in the rumen (Shingfield et al., 2010). The PC3 had also

high loadings on some FA of microbial origin. The OBCFA profile has been proposed as useful tool to predict shifts in microbial population associated in particular with the diet (Vlaeminck et al., 2006). PC6 showed the largest loadings for PUFA n -3, C18:2 n -6, C18:1 $trans$ -11, and C18:2 cis -9, $trans$ -11, i.e., the substrates (the first two) and products (the last two) of the ruminal FA biohydrogenation. Thus, based also on the opposite loading sign for substrates and products, PC6 could be considered as an indicator of PUFA ruminal biohydrogenation activity. The PC8 had large positive loadings on C14:0, C18:1 $trans$ -4, 18:1 $trans$ -16+ cis -14, and negative on C16:1 $trans$ -9, C18:1 $trans$ -11, C18:2 n -6, C18:2 cis -9, $trans$ -11, C20:3 n -6 and C20:4 n -6 (negatives). Considering the high loadings exhibited by PUFA n -6 and by the main products of the biohydrogenation of C18:2 n -6 (C18:1 $trans$ -11 and C18:2 cis -9, $trans$ -11), this PCA could be interpreted as an indicator of PUFA n -6 in the diet.

Factor analysis

The suitability of the data set to the theoretical assumptions of the MFA was assessed through the calculation of the Kaiser Measure of Sampling Adequacy (Kaiser MSA). This index estimates the decrease of partial correlations compared to Pearson correlations between the observed variables. In the present work, the MSA parameter was 0.75, close to the value of 0.80 indicated as the optimal threshold for the suitability of a dataset to MFA (Cerny and Kaiser, 1977). This result was similar to previous reports on the use of MFA on milk FA profile (Mele et al., 2016; Correddu et al., 2017). Nine factors able to explain about 80% of the total variance of the system were extracted (Table 2). The pattern of explained variance across the different factors was smoother compared to PC (Table 1).

The communality of original variables was on average 0.81 (± 0.11), similar to the value reported for buffaloes (0.79) (Correddu et al., 2017) and higher than in cattle (0.69) (Conte et al., 2016; Mele et al., 2016). to 0.96 (for C10:0), The two FA with the lowest value of communality (0.54 for C18:2 $trans$ -9, $trans$ -12 and C18:3 n -6) were the same reported in a work on buffaloes (C18:2 $trans$ -9, $trans$ -12 and C18:3 n -6). Therefore in both species these two FA are characterised by

179 about 50% of independent variation. Largest communalities, in agreement with previous studies, have
180 been found for short and medium chain saturated FA (e.g.: C6:0, C8:0, C10:0, C12:0), associated to
181 the first or second latent factor. The high values observed for these FA, and the agreement among
182 studies, confirm that the variability of these FA is mostly related to a unique metabolic pathway,
183 similar among species.

184 The adequateness of the factor model for fitting the FA correlation matrix was confirmed by
185 the simple structure of the rotated pattern (Morrison, 1976). In particular, each factor showed large
186 loadings with few variables and small loadings with the other variables (Table 2), respectively. Each
187 variable had a large loading in only one factor, with only one exception (C16:0). In total, 42 out of
188 49 FA exhibited a loading value ≥ 0.60 , considered as an empirical threshold for declaring a variable
189 associated to a factor (Macciotta et al., 2015).

190 The first latent factor (F1) was positively correlated with short and medium chain FA (apart
191 from C4:0 and C16:0) and negatively with C18:1*cis*-9 and some long chain saturated FA (C20:0,
192 C22:0 and C24:0). Thus, it was considered an index of “*mammary gland activity*”. A peculiarity of
193 F1 is its structural similarity with PC1. A concordance between the results of the first PC and the first
194 factor extracted from the same data set was observed in a study on body conformation traits in cows
195 (Olasege et al., 2019). F1 structure partially agrees with previous studies where it was associated to
196 mammary gland ability to maintain an optimal milk fat fluidity and to the FA neosynthesis (Conte et
197 al., 2016; Correddu et al., 2017; Palombo et al., 2020). The negative loadings of F1 for long chain
198 saturated FA (C20:0, C22:0 and C24:0) was not observed in previous studies. In a recent investigation
199 on Comisana sheep, they were associated to a factor interpreted as ‘Branched fatty acids metabolism’
200 (Palombo et al., 2020). In cows they were associated to a different factor together with other saturated
201 and unsaturated LCFA (Conte et al. 2016; Mele et al., 2016), whereas in buffaloes they characterized
202 a specific factor (Correddu et al., 2017).

203 Being positively associated to the odd, iso, and anteiso FA (except iso C13:0), F2 was named
204 “*OBCFA*”. These FA are almost completely synthesized by rumen microorganisms (Vlaeminck et

205 al., 2006). This result is in agreement with a previous report on sheep (Palombo et al., 2020), whereas
206 two distinct factors associated with OCFA and BCFA were found in cattle and buffaloes (Conte et al.
207 2016; Correddu et al., 2017). The relative milk concentration of these FA depends on the composition
208 of the microbial population (Vlaemink et al., 2006). The diet, especially its forage to concentrate
209 ratio, is one of the main factors affecting the relative abundance of microbial populations. Thus,
210 feeding management could affect the proportions of OCFA and BCFA in milk. Sheep involved in the
211 present study are farmed in the typical Mediterranean semi-extensive systems with pasture as main
212 feeding source (Macciotta et al., 1999; Molle et al., 2007). Under these conditions, forage to
213 concentrate ratio in the diet should be approximately similar in the various flocks and, therefore, also
214 the rumen microbial composition to a certain extent. As consequence, the correlation pattern of all
215 OBCFA is similar, and the underling pathway of variation is summarized in one unique latent factor.

216 Factor three and four were positively associated with all isomers of C18:1 and C18:2
217 originating from the ruminal biohydrogenation (**BH**) of PUFA, with the exception of C18:1*trans*-11
218 (vaccenic acid) and C18:2*cis*-9,*trans*-11 (rumenic acid). In particular F3 was associated with *trans*
219 isomer of C18:1 from the 4th to the 10th position, C18:1*cis*-12 and, to a lesser extent, to C18:2*trans*-
220 9,*trans*-12. F4 was associated with *trans* isomer of C18:1 from the 13th to the 16th position, C18:2*cis*-
221 9,*trans*-12, C18:2*cis*-9,*trans*-13 and C18:3*cis*-9,*cis*-12,*cis*-15 (C18:3*n*-3, α -linolenic acid, LNA).
222 Although it is very difficult to unequivocally ascertain the metabolic origin of a specific minor BH
223 intermediate (Shingfield et al., 2010), the separation of these FA into two different latent factors can
224 suggest different metabolic pathways underling the BH of PUFA. In particular, FA associated to the
225 3th factor are often produced in the rumen during the BH process of C18:2*cis*-9,*cis*-12 (C18:2*n*-6,
226 linoleic acid) (Shingfield et al., 2010). This result is in agreement with a previous report in cattle
227 where an association of C18:2*n*-6 and its intermediate products in the same latent factor was found
228 (Mele et al., 2016). In the present study C18:2*cis*-9,*cis*-12 was not associated to F3 and, consequently,
229 we decide to assign the generic name of “*biohydrogenation*”. Considering the association of C18:3*n*-
230 3 and of some its ruminal BH intermediates with the F4, this factor was named “*LNA-BH*”. Almost

all FA here found to be associated to F3 and F4 were found in a single latent factor, together with vaccenic and rumenic acids, in previous studies on cattle, buffalo and sheep (Conte et al., 2016; Correddu et al., 2017; Palombo et al., 2020).

The fifth latent factor was named “*desaturase*”, being positively associated with some products of Stearoyl Coenzyme-A Desaturase (SCD) activity (C12:1*cis*-9, C14:1*cis*-9, C16:1*cis*-9 and C17:1*cis*-9) and negatively with the preferred substrate of this enzyme (C18:0). The other SCD products, C18:1*cis*-9 and C18:2*cis*-9,*trans*-11, were highly correlated with the 1st and 7th latent factors, respectively. This result is in agreement with previous investigations in buffaloes (Correddu et al., 2017) and, partially, in cattle (Conte et al., 2016, Mele et al., 2016), where the C17:1*cis*-9 was not associated with the factor related to SCD activity, but with the same factor including C18:1*cis*-9. Results of the present study are also in partial agreement with a previous report in sheep (Palombo et al., 2020). However, in this study the C17:1*cis*-9 did not correlated with any factor. Interestingly, *desaturase* factor presented high loading value for C4:0 (-0.63), differently to previous studies where this FA was associated to a factor with C6:0 (Mele et al., 2016), or was not associated with any factor (Conte et al., 2016; Correddu et al., 2017).

Factor six was named CLA as it showed large correlations with C18:2*cis*-9,*trans*-11 (rumenic acid) and C18:1*trans*-11 (vaccenic acid). It was associated to synthesis of the most abundant and important milk CLA isomer (C18:2*cis*-9,*trans*-11) operated by the SCD in mammary gland. Rumenic and vaccenic acids are of great importance for the nutritional quality of milk (Banni et al., 2003) and many researches have been aimed to find strategies for increasing their concentration (Chilliard et al., 2001; Nudda et al., 2014). High *CLA* factor scores indicate milk characterized by high nutritional value, probably related to sheep grazing high quality pasture. The partition of the SCD products into three different factors is in agreement with the work of Mele et al. (2016), which explained this result with the chain length and the unsaturation degree of the substrate on SCD activity. Conversely, rumenic and vaccenic acids were associated to the biohydrogenation factor in Comisana sheep (Palombo et al. (2020). In the present study also C16:1*trans*-9 was correlated to the *CLA* factor. A

similar result, even though to a lesser extent, was reported in Mele et al. (2016). In another work, it was correlated with the factor associated to the LCFA (Conte et al., 2016).

The seventh and eighth latent factors were named “*n-3*” and “*n-6*” as they were positively correlated with FA of the PUFA $n-3$ family and of the PUFA $n-6$ family, respectively. The extraction of two different factors for PUFA $n-3$ and $n-6$ is in agreement with recent report of buffaloes (Correddu et al., 2017), whereas in cattle they were associated to a unique latent factor (Conte et al., 2016; Mele et al., 2016). This result could arise from differences in the metabolism of these FA, in particular to the capacity to promote C18:3 $n-3$ and C18:2 $n-6$ elongation, or to differences in the dietary concentration of these two FA (Correddu et al., 2016). Although their milk concentration is not high (0.5% of total FA, $n-3$ + $n-6$ excluding C18:3 $n-3$ and C18:2 $n-6$), these FA have great nutritional importance (Connor, 2000). In particular high concentrations of PUFA along with a low $n-6$ to $n-3$ ratio is considered important for good health and normal development in humans (Simopoulos, 2002). The ninth factor explained the 3% of the total variance and did not showed significant loading values.

Mixed model analysis

Results of the mixed-model analysis carried out on the individual scores of the nine PC and of the nine extracted factors are reported in Table 3.

Principal components

On average, the contribution of the flock to the PC variance was around 46%, with the highest values exhibited by PC3 (69%) and the lowest by PC8 (31%). The high contribution of the flock to the variance of PC3 could arise from the great influence of environmental factors as diet, climate and farming practices on ruminal microbial environment (Henderson et al., 2015), which, in turn, influences FA biohydrogenation process and the production of OBCFA. For similar reasons a low

contribution of flock for the PC8 variance was not expected, being this PC interpreted as an indicator of PUFA_{n-6} in the diet.

The DIM class significantly affected e PC1, PC2, and PC9 (Table 3). LS means of PC1 scores exhibited an increasing trend across lactation stages (Figure 2). This trend underlines a reduction in de novo FA synthesis as the lactation proceeds (they have negative loadings) together with an increase of C18:1_{cis}-9 synthesis, in agreement with the reports of Timmen and Patton (1988). The same trend could be observed for PC9, even if the loadings of this PC were very lower compared to PC1. PC2 showed an opposite pattern (Figure 2).

Parity affected significantly PC1, PC5, PC6, and PC8. First lambing ewes exhibited the largest LSmean of PC1 scores (Table 4), that was statistically different from later parities. The PC5 scores decreased across parities, even if with some fluctuations. Scores of PC6 decreased from the 1st to the 5th parity and then increased till the 7th; whereas PC8 showed the opposite behavior (Table 4). Interestingly, the effect of parity on PC6 underline a high concentration of both n-3 and n-6 PUFA in primiparous sheep, followed by a decrease in the intermediate parities and then by an increase in the last parities. Similarly to other milk composition traits, FA are affected by parity due to changes in energy and overall metabolism of the ewes as the lactation number proceeds (González-García et al., 2015). Results of the present study partially agree with previous researches that found higher proportions of more desirable FA in milk of first-parity compared to later parities both in sheep and cows. (Mierlita et al., 2011; Bilal et al., 2014). The larger content of favorable FA especially in first parity animals is conformed also pattern of PC8 scores (Table 4).

The lambing month significantly affected PC1, PC5, PC6, and PC9. Scores for all these PC, except from PC6 (Figure 3), were negative from October to December and positive from January to March. PC1 exhibited larger absolute values in comparison to PC5 and PC9. Altitude of location of flock affected only PC9 scores, with a decreasing trend passing from plain to mountain. The lambing type did not affect any of the 9 PC.

308 *Latent factors*

309 Results of the mixed-model analysis factor scores are reported in Table 3. On average, the
310 contribution of the flock effect to the total variance was 45%, with the highest values for the *n-3*
311 (55%) and the lowest for the *desaturase* (25%) factors, respectively. This finding is consistent with
312 the larger effect of environmental and management factors on the milk content of FA arising from
313 the diet (i.e: PUFA) compared to those of endogen production (i.e.: MUFA produced by delta-9
314 desaturase) (Stoop et al., 2008; Correddu et al., 2019). According to the high value observed for PC3,
315 the *OBCFA* and *biohydrogenation* factors exhibited high values of variance explained by the flock
316 effect (0.49 and 0.53, respectively).

317 Lambing type and the altitude of flock location did not affect any of the extracted factors.
318 The DIM significantly affected *mammary activity*, *OBCFA*, *LNA-BH*, *desaturase*, and *CLA* factor
319 scores. In particular least squares means for scores of *mammary activity*, *LNA-BH* , and *CLA*
320 decreased along the lactation, whereas *OBCFA* and *desaturase* exhibited an opposite trend (Figure
321 4). The effect of DIM class on the *mammary activity* factor confirmed results obtained for PC1. The
322 higher contents of *de novo* FA and lower of C18:1*cis*-9 in early compared to late lactation evidenced
323 by F1 pattern (Figure 4) are in agreement with previous reports in buffaloes (Correddu et al., 2017).
324 On dairy cows a different behavior was observed (Conte et al., 2016; Mele et al., 2016). Such
325 differences could be partially ascribed to differences in the metabolism among species, even if the
326 data distribution along the lactation should be also considered. In the typical Mediterranean sheep
327 farming system, the milk of the first month of lactation is suckled by the lamb. Thus, milk tests
328 considered in the present work were available only from 45 days after parturition, The lack of data
329 for the first month could have therefore hampered the modeling of a trend of FA metabolic pathway
330 in early lactation. Lactation patterns of *LNA-BH* and *CLA* factors evidenced a trend similar to
331 *mammary gland activity*. Such a decreasing pattern underlined a higher activity of LNA ruminal
332 biohydrogenation and of CLA synthesis (due to the increase of SCD substrate, C18:1*trans*-11) in the
333 first part of lactation compared to the last part. This finding was in agreement to that observed for the

334 PC2, and it could be explained by the high content of C18:3 n -3 in spring Mediterranean pastures
335 (Cabiddu et al., 2005), that tends to decrease as in late spring-summer. The pattern of the *Desaturase*
336 factor underlines an increasing SCD activity as the lactation proceeds, as observed in cattle and
337 buffaloes (Mele et al., 2016; Correddu et al., 2017). According to Mele et al. (2016), the increasing
338 trend of *OBCFA* factor along the lactation can be related to the variation of forage to concentrate
339 ratio. An higher amount of concentrate is usually provided in early lactation to meet energy needs of
340 the animals; as the lactation proceeds, there is an increase of the proportion of forages in the diet
341 resulting an increase of FA produced by the ruminal microorganism, in particular by cellulolytic
342 bacteria (Vlaemink et al., 2006). Higher scores for BCFA factor were observed in cows fed a diet
343 with higher percentage of forage (Conte et al., 2016).

344 Parity had significant effect on *mammary activity*, *OBCFA*, n -3, and n -6. *Mammary activity*
345 exhibited an increasing trend from 1st to third parity (Table 5) and then decreased till the eight parity.
346 *OBCFA* scores were rather constant from the 1st to the 4th parity and then rapidly decrease in the 7th
347 and 8th parities. The n -3 and n -6 factors showed a similar waving pattern (Table 5). There is a lack of
348 consensus on the effect parity on latent factors extracted from milk FA. Some works evidenced a
349 large effect (Mele et al., 2016), others minor or no effect (Conte et al., 2016; Correddu et al., 2017).
350 The effect of parity on milk FA is mainly due to the larger PUFA content in primiparous compared
351 to pluriparous animals, that exhibit higher amount of SFA. These figures have been observed both in
352 cows and sheep (Mierlita et al., 2011; Bilal et al., 2014). Differences between parities in the extent of
353 tissue mobilization and in the content of FA synthase in the mammary gland, as well as the rumen
354 microflora, can partially explain the effect of parity on milk FA (Miller et al., 2006; Friggens et al.,
355 2007). In the present work, first lambing animals exhibited lower scores for *mammary activity*, and
356 higher for n -3 and n -6 factors, respectively. Scores of the *OBCFA* factor underlined a decreasing
357 pattern of ruminal derived FA with age, as previously reported in cows and buffaloes (Mele et al.,
358 2016; Correddu et al., 2017).

359 The month of lambing influenced significantly ($P<0.05$) all the latent factors, except from
360 *desaturase* and *n-3*. *Mammary activity*, *LNA-BH*, and *CLA* factors exhibited positive scores for
361 lambings occurring from October to December and negative scores for those from January to March,
362 respectively (Figure 3). An opposite trend could be observed for *OBCFA*, *biohydrogenation*, and *n-*
363 6. Sheep lambing is strictly seasonal, thus the evaluation of the effect of lambing month on a
364 productive response has a different meaning in comparison, for example, with dairy cattle.

365 In the typical farming system of Sarda sheep there is a confounding between lambing season,
366 production season, and parity. Pluriparous ewes lamb in late fall-early winter, whereas first parity
367 animals lamb in late winter-early spring. All the animals are then dried off at the beginning of
368 summer. As a consequence, the number of autumn lambing ewes is larger, and they have also longer
369 lactations. Autumn lambing sheep were sampled in late-lactation, whereas winter lambing sheep were
370 sampled in mid-lactation. Thus, the effects on FA profile of the physiological condition of the animal
371 (stage of lactation, parity) and of the environment (mainly pasture quality) on the FA profile are
372 difficult to disentangle. For example, the larger scores for *mammary activity* found in autumn lambing
373 sheep reflect the higher activity of mammary gland in the FA synthesis in late lactation, whereas
374 winter lambing sheep showed higher content of FA derived from body reserve mobilization in early
375 lactation to meet energy requirement. The lower scores of *LNA-BH* and *CLA* factors observed in milk
376 of sheep lambing in winter underlines a lower activity of rumen LNA biohydrogenation, that result
377 in low milk contents of alpha-linolenic acid, its biohydrogenation intermediates, C18:1*trans*-11 and
378 C18:2*cis*-9,*trans*-11. This pattern reflects, probably, the lower quality of pastures in late spring
379 compared to late-winter early-spring. This finding has interesting implications on the quality of milk
380 in relationship to the season of lambing and to the availability of high-quality pasture, evidencing
381 higher content of desired FA in milk of sheep lambed in autumn.

382

383 **Comparison of the two techniques**

384 The comparison of the two different dimension-reduction methods for analyzing the FA
385 profile of sheep milk provided interesting insights for assessing the usefulness of these two
386 multivariate techniques in deciphering complex correlation patterns and in generating new
387 phenotypes that could be further used for management or genetic purposes.

388 The continuous development of analytical technology has remarkably increased the number
389 of potentially detectable FA. Thus, the number of original variables investigated in the present
390 research was larger in comparison with studies carried out some years ago. In many cases, the newly
391 measured FA were probably not distinguishable from other FA in the previous analyses. Instead of
392 being a simple addition of new variables, this increase of system dimensionality may have added
393 further complexity to the correlation structure of FA. Both PCA and MFA were able to summarize
394 the 49 dimensions of the original multivariate system with 9 new axis that accounted for about 80%
395 of the original variance. Some authors suggest that, when the number of original variables is large,
396 PC and factors tend to coincide (Schneeweis and Mathes, 1995). However, in the present study, some
397 differences have been found in the meaning of the extracted variables.

398 In general, PCA structures were difficult to interpret, also in comparison with previous
399 researches on milk FA profile. On the other hand, in spite of the large number of starting variables,
400 MFA was able to identify through the factor pattern rotation a clear structure of the extracted latent
401 variables. In particular, it was confirmed the ability of this technique, to group FA according to their
402 function or metabolic origin. In agreement with previous works carried out also in other ruminant
403 species, MFA identified key pathways of the milk FA metabolism, as mammary gland de novo
404 synthesis, ruminal biohydrogenation, desaturation performed by SCD enzyme, and rumen microbial
405 activity, that control a relevant quota (80%) of the complex correlation pattern among individual FA.

406 Some partial concordances between the two techniques have been observed. Both PC1 and F1
407 were related to the FA of mammary origin, and the correlation between their scores (Table 6) was
408 rather large (about -0.80). A latent variable related to mammary gland activity able to explain the
409 largest amount of variance was obtained also in other studies (Mele et al., 2016; Palombo et al., 2020).

410 These results suggest to hypothesize a role of main driving force in regulating milk FA (co)variance
411 pattern for mammary FA synthesis pathway. Other large correlations were observed between F9 and
412 PC9 (-0.87), *Biohydrogenation* factor and PC3 (-0.76), *n-3* factor and PC7 (-0.66). This amount of
413 covariation among principal components and factors arise from the fact that both techniques start
414 from the factorization of the correlation matrix. On the other hand, differences still remain due to the
415 different assumptions on the covariance of the system. This fact, together with the possibility of
416 rotating the factor pattern to improve its interpretation, provides more power to the MFA in
417 identifying the real dimensions of milk FA profile system.

418 PCA confirmed its ability in reducing the dimension of the system, but it was not able to
419 efficiently discriminate observations. It has to be considered that the animal sample of the present
420 study was taken from commercial flocks where no specific experimental treatments were applied.
421 Previous studies where PCA was able to distinguish clusters of observations were usually feeding
422 trials where experimental diets aimed at modifying milk FA composition were tested. These
423 treatments may have therefore enhanced differences between animals and emphasised the clustering
424 of observations in the PC space.

425 A major criticism to MFA is for the indeterminacy of its solutions and for the lack of
426 robustness against outliers (Wang et al., 2017). However, it should be pointed out that the various
427 studies on the use of MFA for analysing milk FA, carried out in different species, and under different
428 experimental conditions, led to very similar results. Such a consistency across studies could be
429 considered as a proof for the adequacy of the MFA model to fit the covariance structure of milk FA
430 composition.

431 Individual scores of latent factors extracted from the correlation matrix of FA were able to
432 discriminate cows farmed in herds with different feeding management (Mele et al., 2016). They could
433 be therefore used as synthetic indicators of milk FA metabolism for management purposes. Moreover,
434 genetic parameters of latent factors have been estimated in dairy cattle (Cecchinato et al., 2019). Some
435 latent variables, as the one related to the activity of the SCD factor, showed moderate heritability

(0.31), thus suggesting a possible use of factor scores as novel phenotypes in breeding plans. Instead of being considered simple traits, factor scores should be regarded as aggregate phenotypes and their inclusion as breeding goals should be aimed at improving milk nutritional quality through the modification of specific metabolic pathways.

CONCLUSIONS

The two multivariate statistical techniques used in this study were able to efficiently summarize the milk FA profile of sheep with a reduced number of new variables. However, due to the partitioning of the variance in a large number of extracted variables, PCA was not able to distinguish stratification in the considered sample of animals. On the other hand, the multivariate factor analysis revealed the existence of latent factors controlling the correlation pattern of milk fatty acids. In particular, some independent factors were associated to metabolic pathways involved in the synthesis and modification of milk FA, both in the mammary gland and in the rumen. Moreover, essential FA of dietary origin (PUFA n -3 and PUFA n -6) were associated to two independent factors, confirming the diet as important factor in affecting milk FA profile. The results of the mixed linear model showed a weak influence of the fixed effects on the extracted factors. The clear meaning of the extracted latent factors suggest to hypothesise a possible role as novel phenotypes for breeding and management purposes.

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573

| Descrizione | Mean | SD | CV(%) | Min | Max |
|--------------------------|-------|------|--------|-------|-------|
| C4:0 | 2.67 | 0.37 | 13.83 | 1.52 | 4.05 |
| C6:0 | 1.75 | 0.37 | 21.02 | 0.46 | 2.65 |
| C8:0 | 1.60 | 0.46 | 28.46 | 0.28 | 2.84 |
| C10:0 | 5.52 | 1.76 | 31.86 | 0.87 | 10.18 |
| C10:1 | 0.02 | 0.01 | 51.71 | 0.00 | 0.06 |
| C11:0 | 0.25 | 0.09 | 34.30 | 0.05 | 0.65 |
| C12:0 | 3.48 | 1.00 | 28.78 | 1.08 | 8.15 |
| iso C13:0 | 0.03 | 0.01 | 34.04 | 0.01 | 0.08 |
| C12:1 | 0.04 | 0.01 | 33.41 | 0.02 | 0.13 |
| iso C14:0 | 0.13 | 0.04 | 33.41 | 0.04 | 0.33 |
| C14:0 | 10.81 | 1.54 | 14.23 | 5.28 | 18.42 |
| iso C15:0 | 0.31 | 0.07 | 23.79 | 0.11 | 0.66 |
| anteiso C15:0 | 0.54 | 0.11 | 20.81 | 0.21 | 0.91 |
| C14:1c9 | 0.20 | 0.08 | 42.43 | 0.04 | 0.68 |
| C15:0 | 1.17 | 0.18 | 15.36 | 0.57 | 2.37 |
| iso C16:0 | 0.34 | 0.07 | 20.73 | 0.08 | 0.65 |
| C16:0 | 25.95 | 2.97 | 11.43 | 18.51 | 36.69 |
| iso C17:0 | 0.44 | 0.09 | 19.99 | 0.14 | 0.80 |
| C16:1trans-9 | 0.20 | 0.10 | 48.97 | 0.06 | 0.73 |
| anteiso C17:0 | 0.49 | 0.08 | 17.19 | 0.15 | 0.78 |
| C16:1cis-9 | 0.89 | 0.26 | 29.01 | 0.41 | 2.30 |
| C17:0 | 0.78 | 0.11 | 14.46 | 0.42 | 1.32 |
| C17:1cis-9 | 0.23 | 0.06 | 25.30 | 0.11 | 0.61 |
| C18:0 | 10.29 | 2.51 | 24.38 | 1.37 | 21.00 |
| C18:1trans-4 | 0.02 | 0.01 | 49.99 | 0.00 | 0.16 |
| C18:1trans-5 | 0.02 | 0.01 | 53.52 | 0.00 | 0.12 |
| C18:1trans-6 + 8 | 0.23 | 0.11 | 49.45 | 0.07 | 1.10 |
| C18:1trans-9 | 0.27 | 0.08 | 31.56 | 0.13 | 0.91 |
| C18:1trans-10 | 0.42 | 0.44 | 105.73 | 0.11 | 7.85 |
| C18:1trans-11 | 2.06 | 1.03 | 50.21 | 0.46 | 5.77 |
| C18:1trans-13 + trans-14 | 0.86 | 0.45 | 51.90 | 0.22 | 4.74 |
| C18:1c9 | 17.23 | 3.64 | 21.11 | 5.37 | 34.75 |
| C18:1cis-12 | 0.31 | 0.13 | 40.17 | 0.11 | 1.07 |
| C18:1trans-16 + c14 | 0.50 | 0.15 | 29.34 | 0.12 | 1.08 |
| C18:2trans-9,trans-12 | 0.02 | 0.01 | 63.00 | 0.01 | 0.18 |
| C18:2cis-9,trans-13 | 0.44 | 0.17 | 38.08 | 0.14 | 1.64 |
| C18:2cis-9,trans-12 | 0.15 | 0.03 | 23.38 | 0.07 | 0.34 |
| C18:2n6 | 2.09 | 0.51 | 24.33 | 0.92 | 4.32 |
| C20:0 | 0.32 | 0.12 | 39.19 | 0.04 | 1.36 |
| C18:3n6 | 0.04 | 0.02 | 39.81 | 0.01 | 0.15 |
| C18:3n3 | 0.89 | 0.50 | 55.76 | 0.20 | 3.35 |
| C18:2cis-9,trans-11 | 1.03 | 0.47 | 45.52 | 0.28 | 3.16 |
| C22:0 | 0.17 | 0.06 | 32.76 | 0.02 | 0.50 |
| C20:3n6 | 0.03 | 0.01 | 29.32 | 0.01 | 0.07 |
| C20:4n6 | 0.13 | 0.05 | 36.62 | 0.04 | 0.33 |
| EPA | 0.06 | 0.02 | 29.91 | 0.03 | 0.15 |
| C24:0 | 0.08 | 0.03 | 40.23 | 0.00 | 0.19 |
| DPA | 0.13 | 0.03 | 27.05 | 0.04 | 0.28 |
| DHA | 0.04 | 0.02 | 38.70 | 0.01 | 0.12 |

576 **Table 1.** Eigenvectors and eigenvalues of the first nine principal components extracted from the
577 correlation matrix of the 49 Fatty acids.

| | Principal Component (PC) | | | | | | | | |
|-----------------------|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 |
| C4:0 | -0.054 | 0.143 | 0.006 | -0.205 | 0.123 | 0.041 | -0.287 | -0.160 | 0.099 |
| C6:0 | -0.219 | 0.039 | 0.119 | -0.099 | 0.239 | 0.031 | -0.036 | -0.062 | 0.054 |
| C8:0 | -0.233 | 0.009 | 0.124 | -0.033 | 0.237 | 0.011 | 0.052 | -0.047 | 0.013 |
| C10:0 | -0.239 | -0.044 | 0.124 | 0.015 | 0.216 | -0.005 | 0.110 | -0.006 | 0.036 |
| C10:1 | -0.189 | -0.074 | 0.044 | 0.101 | 0.095 | -0.035 | 0.103 | 0.116 | 0.005 |
| C11:0 | -0.201 | -0.173 | 0.102 | 0.167 | 0.068 | -0.010 | 0.042 | 0.056 | -0.048 |
| C12:0 | -0.228 | -0.094 | 0.109 | 0.068 | 0.190 | -0.022 | 0.151 | 0.045 | 0.019 |
| isoC13:0 | 0.192 | 0.019 | 0.113 | -0.114 | -0.042 | -0.072 | 0.099 | 0.137 | 0.143 |
| anteisoC13:0 | -0.094 | -0.246 | 0.071 | 0.217 | -0.029 | -0.051 | 0.096 | 0.181 | -0.032 |
| isoC14:0 | 0.198 | -0.008 | 0.155 | 0.096 | 0.054 | -0.100 | -0.065 | 0.013 | 0.281 |
| C14:0 | -0.170 | -0.206 | 0.092 | 0.005 | 0.011 | -0.021 | 0.091 | 0.198 | 0.174 |
| isoC15:0 | 0.210 | 0.044 | 0.134 | 0.030 | 0.024 | -0.213 | 0.033 | 0.063 | 0.004 |
| anteisoC15:0 | 0.090 | 0.128 | 0.198 | 0.193 | 0.101 | -0.278 | -0.057 | 0.041 | -0.027 |
| C14:1cis-9 | -0.011 | -0.288 | -0.010 | 0.188 | -0.199 | -0.010 | -0.022 | 0.172 | -0.008 |
| C15:0 | 0.019 | 0.049 | 0.224 | 0.275 | 0.040 | -0.098 | -0.019 | 0.110 | 0.146 |
| isoC16:0 | 0.151 | 0.048 | 0.130 | 0.186 | 0.180 | -0.137 | -0.149 | 0.059 | 0.136 |
| C16:0 | 0.038 | -0.245 | -0.001 | -0.118 | -0.199 | 0.087 | -0.147 | 0.031 | 0.249 |
| isoC17:0 | 0.214 | 0.035 | -0.035 | 0.092 | 0.183 | -0.131 | 0.032 | 0.025 | -0.207 |
| C16:1trans-9 | -0.114 | 0.213 | 0.023 | 0.106 | -0.202 | -0.180 | 0.077 | -0.311 | 0.147 |
| anteisoC17:0 | 0.127 | 0.105 | 0.096 | 0.241 | 0.249 | -0.148 | -0.060 | -0.014 | -0.211 |
| C16:1cis-9 | 0.039 | -0.248 | -0.024 | 0.194 | -0.289 | 0.018 | -0.108 | 0.036 | -0.103 |
| C17:0 | 0.126 | 0.052 | 0.212 | 0.205 | 0.127 | 0.120 | 0.088 | 0.014 | 0.037 |
| C17:1cis-9 | 0.133 | -0.103 | 0.076 | 0.281 | -0.147 | 0.032 | -0.022 | -0.083 | -0.196 |
| C18:0 | 0.155 | 0.191 | -0.021 | -0.212 | 0.160 | -0.078 | 0.107 | 0.109 | -0.158 |
| C18:1trans-4 | 0.096 | 0.030 | -0.246 | -0.041 | 0.107 | -0.015 | 0.245 | 0.202 | 0.147 |
| C18:1trans-5 | 0.054 | 0.027 | -0.263 | 0.031 | 0.119 | 0.007 | 0.274 | 0.117 | 0.185 |
| C18:1trans-6+8 | 0.030 | 0.038 | -0.344 | 0.106 | 0.060 | -0.087 | 0.147 | 0.056 | 0.116 |
| C18:1trans-9 | 0.025 | 0.064 | -0.339 | 0.107 | 0.002 | -0.121 | 0.121 | 0.008 | 0.067 |
| C18:1trans-10 | -0.007 | -0.013 | -0.245 | 0.194 | 0.086 | -0.003 | 0.093 | -0.066 | 0.131 |
| C18:1trans-11 | -0.122 | 0.233 | -0.033 | 0.104 | -0.138 | -0.214 | 0.081 | -0.263 | 0.186 |
| C18:1trans-13+t14 | -0.154 | 0.216 | -0.080 | 0.125 | 0.088 | 0.117 | -0.154 | 0.156 | 0.001 |
| C18:1cis-9 | 0.229 | -0.018 | -0.089 | -0.012 | -0.100 | -0.030 | -0.012 | -0.059 | -0.336 |
| C18:1cis-12 | 0.071 | -0.043 | -0.294 | 0.095 | 0.126 | 0.089 | -0.090 | 0.032 | 0.037 |
| C18:1trans-16+cis-14 | -0.090 | 0.284 | -0.073 | 0.056 | 0.064 | 0.117 | -0.160 | 0.210 | -0.128 |
| C18:2trans-9,trans-12 | -0.030 | 0.013 | -0.159 | 0.253 | 0.033 | 0.152 | 0.031 | 0.001 | 0.205 |
| C18:2cis-9,trans-13 | -0.139 | 0.162 | -0.101 | 0.253 | -0.091 | 0.119 | -0.166 | 0.124 | -0.174 |
| C18:2cis-9trans-12 | -0.087 | 0.192 | -0.139 | 0.190 | -0.012 | 0.143 | -0.197 | 0.176 | -0.121 |
| C18:2n-6 | 0.093 | -0.056 | -0.063 | 0.149 | 0.133 | 0.312 | -0.249 | -0.268 | 0.134 |
| C20:0 | 0.245 | 0.003 | 0.010 | -0.020 | -0.015 | 0.034 | -0.018 | 0.157 | 0.172 |
| C18:3n-6 | 0.020 | -0.205 | -0.001 | 0.076 | 0.193 | 0.118 | -0.103 | -0.150 | 0.125 |
| C18:3n-3 | -0.105 | 0.212 | 0.105 | 0.015 | -0.150 | 0.289 | -0.066 | 0.072 | 0.129 |
| C18:2cis-9,trans-11 | -0.111 | 0.150 | -0.027 | 0.193 | -0.267 | -0.224 | 0.076 | -0.306 | 0.085 |
| C22:0 | 0.205 | 0.114 | 0.119 | 0.019 | -0.070 | 0.102 | -0.102 | 0.142 | 0.267 |
| C20:3n-6 | 0.144 | -0.121 | -0.044 | 0.090 | 0.213 | 0.131 | 0.001 | -0.280 | 0.027 |
| C20:4n-6 | 0.153 | -0.160 | -0.019 | 0.064 | 0.193 | 0.141 | 0.059 | -0.326 | -0.077 |
| EPA | -0.039 | 0.176 | 0.169 | 0.088 | -0.104 | 0.259 | 0.277 | -0.004 | -0.028 |
| C24:0 | 0.189 | 0.147 | 0.127 | -0.002 | -0.066 | 0.118 | -0.070 | 0.092 | 0.205 |
| DPA | 0.090 | 0.137 | 0.150 | 0.064 | -0.069 | 0.299 | 0.367 | -0.072 | -0.087 |
| DHA | 0.120 | 0.044 | 0.098 | 0.022 | -0.052 | 0.313 | 0.346 | -0.043 | -0.081 |
| eigenvalues | 12.28 | 7.38 | 6.55 | 3.84 | 2.61 | 2.58 | 1.53 | 1.42 | 1.26 |
| Var. explained (%) | 25.06 | 15.06 | 13.37 | 7.83 | 5.32 | 5.27 | 3.13 | 2.89 | 2.57 |

| | Factors ¹ | | | | | | | | | Com ² |
|---|----------------------|-------------|-------------|-------------|--------------|-------------|-------------|-------------|-------------|------------------|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | |
| C12:0 | 0.95 | -0.06 | -0.11 | 0.03 | 0.06 | 0.02 | -0.01 | -0.06 | -0.03 | 0.94 |
| C10:0 | 0.95 | -0.08 | -0.19 | 0.06 | -0.11 | 0.06 | 0.00 | -0.06 | -0.01 | 0.96 |
| C8:0 | 0.87 | -0.09 | -0.24 | 0.12 | -0.28 | 0.07 | -0.01 | -0.05 | -0.03 | 0.93 |
| C11:0 | 0.83 | -0.05 | -0.17 | 0.06 | 0.41 | 0.01 | -0.08 | 0.03 | -0.03 | 0.91 |
| C6:0 | 0.77 | -0.13 | -0.29 | 0.14 | -0.42 | 0.05 | -0.05 | -0.03 | 0.05 | 0.89 |
| C10:1 | 0.73 | -0.06 | 0.00 | 0.12 | 0.17 | 0.04 | -0.05 | -0.12 | -0.01 | 0.59 |
| C14:0 | 0.73 | -0.17 | -0.12 | -0.17 | 0.35 | -0.13 | -0.11 | -0.14 | 0.25 | 0.83 |
| isoC13:0 | -0.48 | 0.36 | -0.08 | -0.41 | -0.08 | -0.20 | 0.18 | -0.18 | 0.17 | 0.68 |
| C24:0 | -0.58 | 0.45 | -0.15 | 0.01 | -0.18 | -0.08 | 0.35 | 0.01 | 0.32 | 0.82 |
| C22:0 | -0.60 | 0.49 | -0.11 | -0.02 | -0.10 | -0.13 | 0.29 | 0.03 | 0.40 | 0.88 |
| C20:0 | -0.66 | 0.37 | 0.14 | -0.25 | 0.02 | -0.31 | 0.13 | 0.07 | 0.21 | 0.82 |
| C18:1 <i>cis</i> -9 | -0.79 | 0.10 | 0.11 | -0.18 | 0.16 | -0.18 | 0.02 | 0.10 | -0.37 | 0.88 |
| anteisoC15:0 | -0.08 | 0.86 | -0.19 | 0.01 | -0.06 | 0.20 | 0.01 | -0.14 | -0.13 | 0.85 |
| isoC16:0 | -0.20 | 0.81 | -0.03 | -0.05 | -0.02 | -0.06 | -0.04 | 0.16 | 0.06 | 0.73 |
| anteisoC17:0 | -0.15 | 0.80 | 0.02 | 0.12 | -0.07 | -0.01 | 0.04 | 0.14 | -0.38 | 0.84 |
| C15:0 | 0.19 | 0.72 | -0.20 | 0.10 | 0.19 | 0.16 | 0.16 | -0.03 | 0.14 | 0.72 |
| isoC14:0 | -0.35 | 0.69 | -0.08 | -0.33 | 0.06 | -0.07 | 0.07 | 0.15 | 0.24 | 0.82 |
| C17:0 | -0.07 | 0.67 | -0.16 | -0.02 | 0.05 | -0.10 | 0.48 | 0.22 | 0.03 | 0.76 |
| isoC15:0 | -0.47 | 0.66 | -0.08 | -0.34 | -0.02 | -0.07 | 0.07 | -0.12 | -0.07 | 0.81 |
| isoC17:0 | -0.48 | 0.53 | 0.26 | -0.14 | -0.05 | -0.22 | 0.00 | 0.11 | -0.37 | 0.80 |
| C18:1 <i>trans</i> -6 + 8 | -0.18 | -0.12 | 0.89 | 0.14 | 0.00 | 0.10 | -0.19 | 0.02 | -0.08 | 0.92 |
| C18:1 <i>trans</i> -9 | -0.23 | -0.14 | 0.83 | 0.17 | 0.00 | 0.21 | -0.21 | -0.02 | -0.13 | 0.90 |
| C18:1 <i>trans</i> -5 | -0.13 | -0.08 | 0.82 | -0.02 | -0.10 | -0.08 | 0.03 | 0.00 | 0.02 | 0.71 |
| C18:1 <i>trans</i> -4 | -0.27 | -0.05 | 0.76 | -0.08 | -0.14 | -0.21 | 0.01 | -0.10 | 0.02 | 0.73 |
| C18:1 <i>trans</i> -10 | 0.04 | -0.06 | 0.68 | 0.15 | 0.13 | 0.15 | -0.11 | 0.25 | -0.05 | 0.60 |
| C18:1 <i>cis</i> -12 | -0.25 | -0.12 | 0.65 | 0.18 | 0.07 | -0.20 | -0.22 | 0.35 | -0.05 | 0.75 |
| C18:2 <i>trans</i> -9, <i>trans</i> -12 | 0.11 | 0.00 | 0.49 | 0.34 | 0.19 | 0.13 | 0.08 | 0.29 | 0.15 | 0.54 |
| C18:2 <i>cis</i> -9, <i>trans</i> -13 | 0.16 | -0.08 | 0.11 | 0.87 | 0.11 | 0.27 | 0.03 | -0.06 | -0.07 | 0.90 |
| C18:2 <i>cis</i> -9, <i>trans</i> -12 | 0.01 | -0.04 | 0.22 | 0.86 | -0.07 | 0.13 | 0.00 | -0.03 | -0.02 | 0.81 |
| C18:1 <i>trans</i> -16 + <i>cis</i> -14 | 0.02 | 0.02 | 0.09 | 0.82 | -0.41 | 0.08 | 0.08 | -0.21 | -0.03 | 0.91 |
| C18:1 <i>trans</i> -13 + <i>trans</i> -14 | 0.29 | -0.03 | 0.14 | 0.80 | -0.29 | 0.17 | 0.01 | -0.09 | 0.07 | 0.86 |
| C18:3 <i>n</i> -3 | 0.09 | -0.11 | -0.30 | 0.56 | -0.23 | 0.21 | 0.43 | -0.12 | 0.36 | 0.85 |
| C14:1 <i>cis</i> -9 | 0.14 | -0.08 | 0.02 | -0.14 | 0.88 | -0.16 | -0.16 | 0.07 | 0.10 | 0.89 |
| C16:1 <i>cis</i> -9 | -0.14 | -0.10 | -0.07 | -0.09 | 0.88 | -0.05 | -0.14 | 0.17 | 0.01 | 0.87 |
| C12:1 <i>cis</i> -9 | 0.55 | 0.06 | -0.02 | -0.10 | 0.71 | -0.12 | -0.08 | 0.00 | 0.00 | 0.84 |
| C17:1 <i>cis</i> -9 | -0.30 | 0.35 | -0.11 | -0.04 | 0.62 | 0.02 | 0.18 | 0.28 | -0.19 | 0.75 |
| C18:0 | -0.50 | 0.22 | 0.13 | -0.10 | -0.61 | -0.23 | 0.13 | -0.27 | -0.23 | 0.89 |
| C4:0 | 0.00 | -0.14 | -0.23 | 0.17 | -0.63 | 0.07 | -0.19 | 0.08 | 0.13 | 0.57 |
| C18:2 <i>cis</i> -9 <i>trans</i> -11 | 0.08 | 0.00 | 0.04 | 0.22 | 0.09 | 0.92 | -0.02 | -0.17 | -0.05 | 0.93 |
| C16:1 <i>trans</i> -9 | 0.10 | 0.02 | -0.05 | 0.21 | -0.17 | 0.88 | 0.07 | -0.19 | 0.03 | 0.91 |
| C18:1 <i>trans</i> -11 | 0.13 | 0.03 | 0.11 | 0.25 | -0.26 | 0.86 | -0.01 | -0.22 | 0.03 | 0.95 |
| DPA | -0.20 | 0.17 | -0.12 | 0.03 | -0.08 | 0.04 | 0.88 | 0.03 | -0.05 | 0.87 |
| DHA | -0.25 | 0.07 | -0.04 | -0.11 | 0.02 | -0.15 | 0.77 | 0.12 | -0.03 | 0.71 |
| EPA | 0.11 | 0.09 | -0.23 | 0.27 | -0.10 | 0.20 | 0.75 | -0.12 | 0.07 | 0.78 |
| C18:2 <i>n</i> -6 | -0.20 | 0.06 | 0.10 | 0.14 | 0.06 | -0.13 | 0.06 | 0.80 | 0.13 | 0.76 |
| C20:4 <i>n</i> -6 | -0.18 | 0.12 | 0.12 | -0.39 | 0.13 | -0.25 | 0.13 | 0.67 | -0.24 | 0.81 |
| C20:3 <i>n</i> -6 | -0.18 | 0.17 | 0.20 | -0.28 | 0.07 | -0.21 | 0.07 | 0.66 | -0.13 | 0.68 |
| C18:3 <i>n</i> -6 | 0.21 | 0.03 | 0.04 | -0.22 | 0.20 | -0.25 | -0.12 | 0.56 | 0.07 | 0.54 |
| C16:0 | -0.05 | -0.07 | -0.04 | -0.04 | 0.06 | 0.00 | -0.07 | 0.04 | 0.42 | 0.75 |
| Eigenvalue | 8.92 | 5.47 | 4.79 | 4.74 | 4.70 | 3.47 | 3.04 | 2.81 | 1.53 | |
| Var. explained (%) | 17.62 | 10.80 | 9.46 | 9.36 | 9.29 | 6.86 | 6.00 | 5.54 | 3.01 | |

580 ¹ F1 = *Mammary activity*; F2 = *OBCFA*; F3= *Biohydrogenation*; F4 = *LNA (alpha-linolenic acid) BH*;
581 F5 = *Desaturase*; F6 = *CLA*; F7 = *n-3*; F8 = *n-6*; F9 = *C16*.
582 ² Communalilty.
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Table 3. Effect of DIM, parity, month and type of lambing, and altitude of flock on the 9 principal components (PC) and 9 latent factors (F)

| item | | <i>P-value</i> | | | | <i>Flock (zone)</i> | |
|-----------------------------|-------------------------|----------------|--------|---------------|--------------|---------------------|----------|
| | | DIM | Parity | Lambing-month | Lambing-type | | Altitude |
| Principal components | | | | | | | |
| PC1 | | <0.001 | <0.001 | <0.001 | 0.683 | 0.469 | 0.53 |
| PC2 | | <0.001 | 0.647 | 0.413 | 0.213 | 0.831 | 0.53 |
| PC3 | | 0.762 | 0.635 | 0.249 | 0.267 | 0.545 | 0.69 |
| PC4 | | 0.067 | 0.157 | 0.072 | 0.934 | 0.407 | 0.36 |
| PC5 | | 0.195 | 0.008 | 0.006 | 0.177 | 0.343 | 0.42 |
| PC6 | | 0.153 | 0.006 | 0.029 | 0.744 | 0.526 | 0.51 |
| PC7 | | 0.187 | 0.180 | 0.469 | 0.079 | 0.156 | 0.39 |
| PC8 | | 0.186 | 0.018 | 0.691 | 0.209 | 0.938 | 0.31 |
| PC9 | | 0.032 | 0.688 | <0.001 | 0.337 | 0.042 | 0.37 |
| Latent factors ¹ | | | | | | | |
| F1 | <i>mammary activity</i> | <0.001 | 0.022 | <0.001 | 0.860 | 0.921 | 0.43 |
| F2 | <i>OBCFA</i> | <0.001 | <0.001 | <0.001 | 0.559 | 0.907 | 0.49 |
| F3 | <i>biohydrogenation</i> | 0.137 | 0.800 | 0.025 | 0.486 | 0.596 | 0.53 |
| F4 | <i>LNA-BH</i> | <0.001 | 0.588 | <0.001 | 0.059 | 0.222 | 0.39 |
| F5 | <i>desaturase</i> | <0.001 | 0.614 | 0.143 | 0.187 | 0.425 | 0.25 |
| F6 | <i>CLA</i> | <0.001 | 0.209 | 0.002 | 0.350 | 0.583 | 0.40 |
| F7 | <i>n-3</i> | 0.062 | 0.001 | 0.213 | 0.140 | 0.445 | 0.55 |
| F8 | <i>n-6</i> | 0.122 | 0.007 | <0.001 | 0.901 | 0.501 | 0.50 |
| F9 | <i>CI6</i> | 0.004 | 0.500 | 0.016 | 0.175 | 0.031 | 0.52 |

¹Flock(zone) = contribute of flock nested within altitude of location to the total variance;

²*OBCFA* = odd and branched-chain fatty acids; *LNA-BH* = alpha-linolenic acid (C18:3*cis*-9,*cis*-12,*cis*-15) biohydrogenation; *CLA* = conjugated linoleic acids; *n-3* = polyunsaturated fatty acids belonging to the omega-3 family; *n-6* = polyunsaturated fatty acids belonging to the omega-6 family; *CI6* = palmitic acid (C16:0).

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Table 4. Least square means (\pm standard error) of the principal components affected by parity.

| parity | <i>Principal component</i> | | | |
|--------|-------------------------------|------------------|--------------------------------|--------------------------------|
| | PC1 | PC5* | PC6 | PC8 |
| 1 | 1.98 ^a \pm 0.45 | 0.54 \pm 0.21 | 0.29 ^a \pm 0.23 | -0.09 ^{ab} \pm 0.15 |
| 2 | 0.60 ^b \pm 0.45 | 0.30 \pm 0.21 | 0.03 ^{ab} \pm 0.23 | 0.10 ^{ab} \pm 0.16 |
| 3 | 0.30 ^b \pm 0.44 | 0.44 \pm 0.21 | -0.26 ^{ab} \pm 0.23 | 0.08 ^{ab} \pm 0.15 |
| 4 | 0.53 ^b \pm 0.44 | 0.34 \pm 0.20 | -0.27 ^b \pm 0.23 | 0.27 ^a \pm 0.15 |
| 5 | 0.47 ^b \pm 0.45 | 0.28 \pm 0.21 | -0.28 ^{ab} \pm 0.23 | 0.07 ^{ab} \pm 0.16 |
| 6 | 0.42 ^b \pm 0.46 | 0.02 \pm 0.22 | -0.03 ^{ab} \pm 0.24 | -0.04 ^{ab} \pm 0.16 |
| 7 | 0.56 ^b \pm 0.49 | -0.03 \pm 0.24 | 0.16 ^{ab} \pm 0.26 | -0.20 ^b \pm 0.18 |
| 8 | 0.49 ^{ab} \pm 0.64 | -0.35 \pm 0.32 | -0.17 ^{ab} \pm 0.34 | -0.32 ^{ab} \pm 0.26 |

^{a,b,c}, least square means with different superscript letters within a column differ (P<0.05)

*although PC5 was significantly affect by parity, differences among contrasts did not reach the statistical significance ($\alpha = 0.05$).

Table 5. Least square means (\pm standard error) of the latent factors affected by parity

| parity | <i>Latent factors</i> | | | |
|--------|--------------------------------|---------------------------------|---------------------------------|--------------------------------|
| | <i>mammary activity</i> | <i>OBCFA</i> | <i>n-3</i> | <i>n-6</i> |
| 1 | -0.37 ^b \pm 0.13 | 0.23 ^{ab} \pm 0.14 | 0.09 ^{ab} \pm 0.14 | 0.35 ^a \pm 0.14 |
| 2 | -0.06 ^{ab} \pm 0.13 | 0.15 ^{ab} \pm 0.15 | -0.03 ^{abc} \pm 0.14 | 0.11 ^{ab} \pm 0.14 |
| 3 | 0.04 ^a \pm 0.13 | 0.23 ^a \pm 0.14 | -0.24 ^c \pm 0.14 | 0.08 ^{ab} \pm 0.13 |
| 4 | -0.04 ^{ab} \pm 0.13 | 0.21 ^a \pm 0.14 | -0.21 ^{bc} \pm 0.14 | -0.07 ^b \pm 0.13 |
| 5 | -0.08 ^{ab} \pm 0.13 | 0.08 ^{abc} \pm 0.15 | -0.15 ^{abc} \pm 0.14 | -0.05 ^b \pm 0.14 |
| 6 | -0.10 ^{ab} \pm 0.14 | -0.01 ^{abc} \pm 0.15 | 0.05 ^a \pm 0.15 | 0.01 ^{ab} \pm 0.14 |
| 7 | -0.16 ^{ab} \pm 0.15 | -0.15 ^{bc} \pm 0.16 | 0.06 ^{abc} \pm 0.16 | 0.15 ^{ab} \pm 0.15 |
| 8 | -0.29 ^{ab} \pm 0.20 | -0.45 ^c \pm 0.21 | -0.14 ^{abc} \pm 0.20 | -0.01 ^{ab} \pm 0.20 |

^{a,b,c}, least square means with different superscript letters within a column differ (P<0.05)

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Table 6 Correlation matrix between the scores of principal components and latent factors

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 |
|-----------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Factor1 Mammary | -0.78 | -0.25 | 0.24 | 0.16 | 0.43 | -0.03 | 0.23 | 0.05 | 0.11 |
| | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.3976 | <0.0001 | 0.1532 | 0.0008 |
| Factor2 OBCFA | 0.41 | 0.21 | 0.45 | 0.51 | 0.37 | -0.36 | -0.07 | 0.18 | 0.13 |
| | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0276 | <0.0001 | <0.0001 |
| Factor3 BH | 0.12 | 0.04 | -0.76 | 0.25 | 0.25 | -0.09 | 0.42 | 0.16 | 0.26 |
| | 0.0002 | 0.2467 | <0.0001 | <0.0001 | <0.0001 | 0.0067 | <0.0001 | <0.0001 | <0.0001 |
| Factor4 LNA BH | -0.32 | 0.50 | -0.20 | 0.40 | -0.05 | 0.35 | -0.43 | 0.30 | -0.23 |
| | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.1463 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Factor5 Desaturase | 0.03 | -0.61 | 0.01 | 0.55 | -0.51 | -0.01 | 0.04 | 0.20 | -0.13 |
| | 0.3162 | <0.0001 | 0.7817 | <0.0001 | <0.0001 | 0.8356 | 0.234 | <0.0001 | <0.0001 |
| Factor6 CLA | -0.26 | 0.37 | -0.02 | 0.29 | -0.42 | -0.36 | 0.11 | -0.60 | 0.22 |
| | <0.0001 | <0.0001 | 0.5706 | <0.0001 | <0.0001 | <0.0001 | 0.0008 | <0.0001 | <0.0001 |
| Factor7 N3 | 0.14 | 0.26 | 0.31 | 0.12 | -0.12 | 0.59 | 0.66 | -0.03 | -0.05 |
| | <0.0001 | <0.0001 | <0.0001 | 0.0001 | 0.0003 | <0.0001 | <0.0001 | 0.3595 | 0.1358 |
| Factor8 N6 | 0.18 | -0.27 | -0.10 | 0.28 | 0.34 | 0.45 | -0.31 | -0.62 | 0.12 |
| | <0.0001 | <0.0001 | 0.0021 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0001 |
| Factor9 | -0.04 | -0.01 | 0.11 | -0.09 | -0.23 | 0.24 | -0.20 | 0.26 | 0.87 |
| | 0.2497 | 0.671 | 0.0004 | 0.0072 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

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621 **Figure Captions**

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624 **Figure 1.** Plots of the scores for the first two principal components (PC1 and PC2) of animals
625 belonging to different class of DIM (from 1 to 5 in figure 1A and averaged in mid and late lactation
626 in figure 2B).

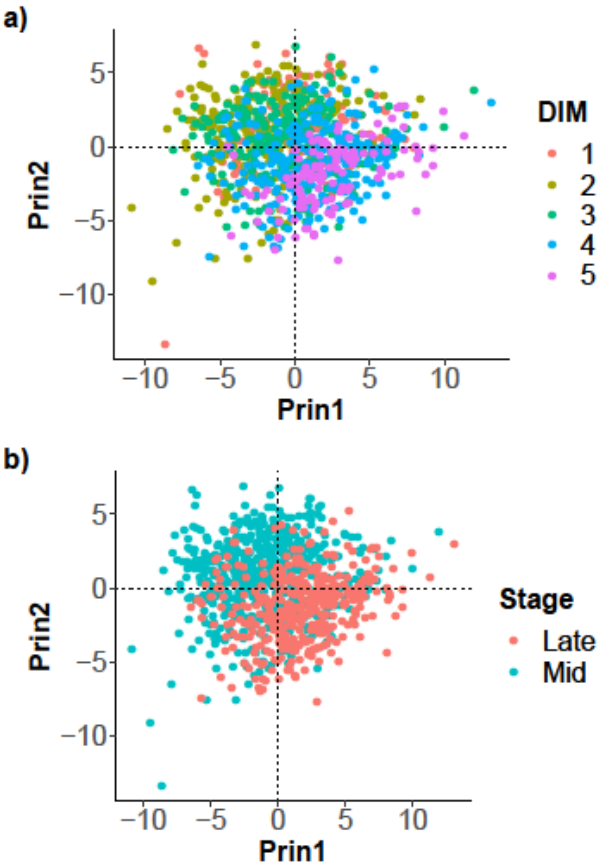
627 **Figure 2.** Classes of days in milk (DIM) pattern of PC1, PC2 and PC9.

628 **Figure 3.** Effect of lambing month on PC1, PC5, PC6 and PC9.

629 **Figure 4.** Classes of days in milk (DIM) pattern of *mammary activity*, *OBCFA*, *LNA-BH*,
630 *Desaturase* and *CLA* factors.

631 **Figure 5.** Effect of lambing month on *mammary activity*, *OBCFA*, biohydrogenation, *LNA-BH*,
632 *CLA* and *n-6* factors.

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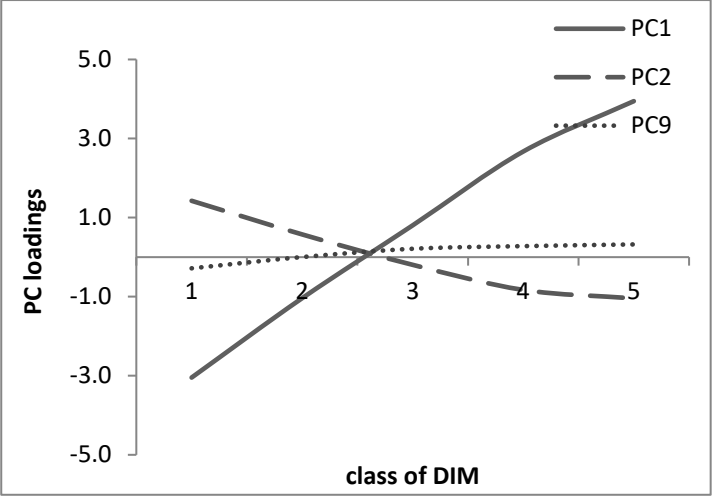
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638 **Correddu. Figure 2.**

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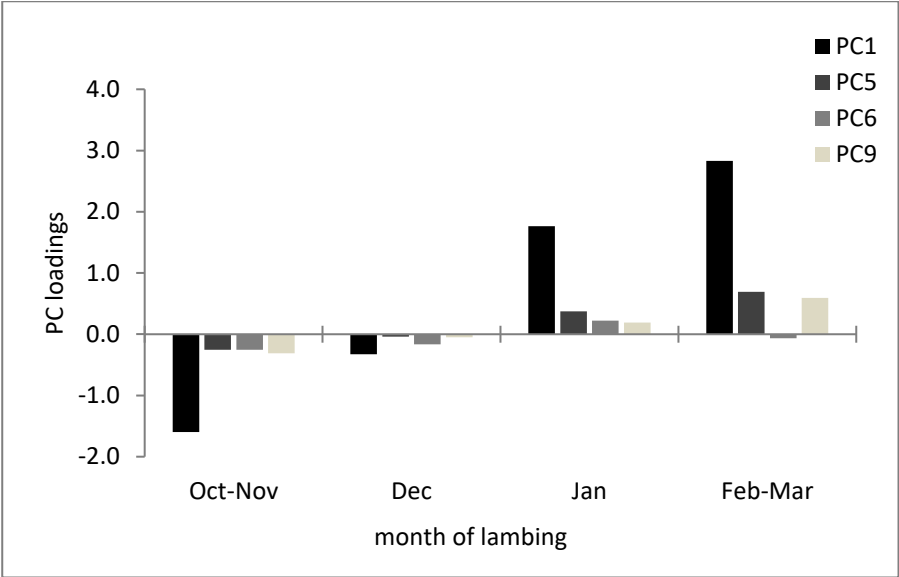
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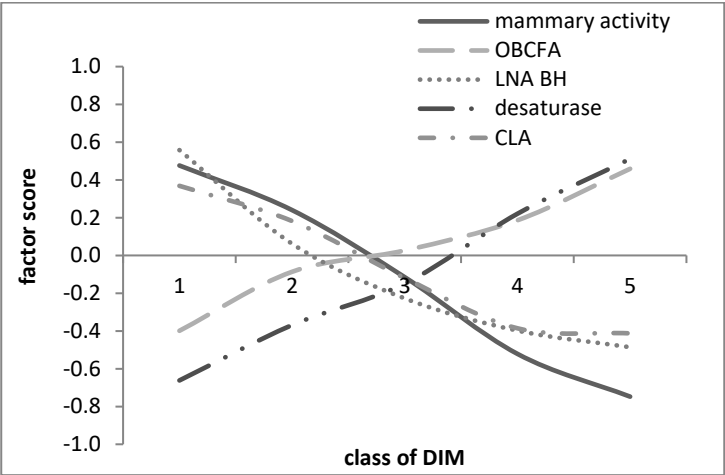
647 **Correddu. Figure 3.**

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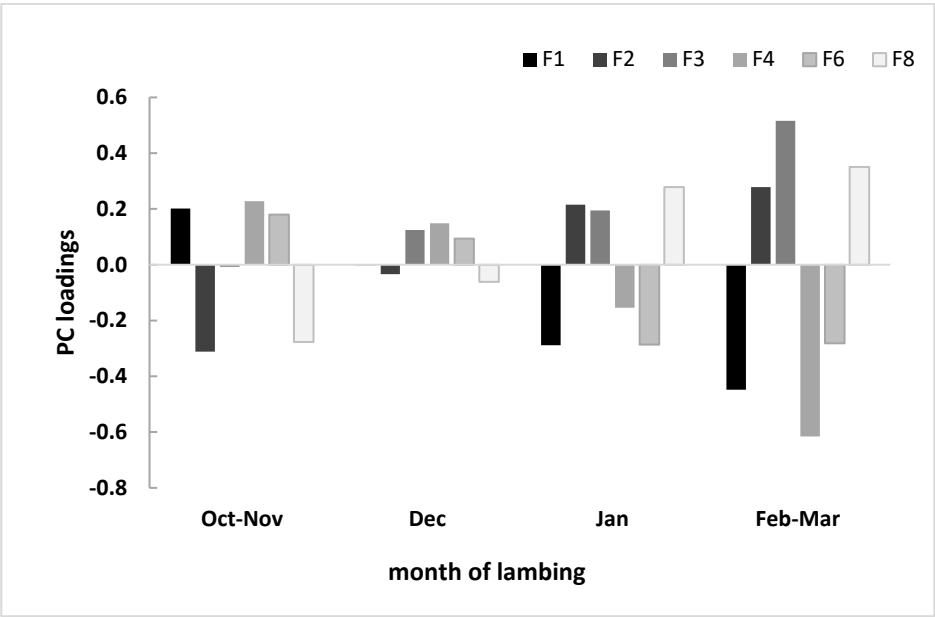
650 **Correddu. Figure 4.**
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654 **Correddu. Figure 5.**

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